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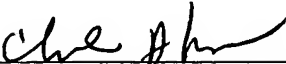
**LETTER**

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Sir:

Supplemental to the letter of August 11, 2008, Applicants are submitting herewith the K-E Andersson et al paper which is the last reference cited in the declaration of Dr. Thurieau which is believed to demonstrate Applicants' invention.

Respectfully submitted,

  
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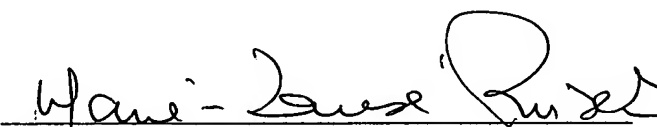
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Marie-Louise Pinset

# New directions for erectile dysfunction therapies

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Research in the field of erectile function and dysfunction has continued to expand rapidly. Based on the information available, some directions for future erectile dysfunction therapies can be identified. The first direction is improvement of current therapeutic principles. A second generation of orally active phosphodiesterase (PDE) inhibitors is being introduced, and further developments within this field can be expected. The recent introduction of apomorphine has opened the way for new dopamine receptor agonists. The second direction is combinations of existing therapeutic principles. Combinations of apomorphine and sildenafil and apomorphine and  $\alpha_1$ -adrenoceptor (AR) antagonists, for example, seem attractive and may have a therapeutic potential in patients not responding satisfactorily to single-drug treatment. Nitrosylated  $\alpha_1$ -AR antagonists, combining nitric oxide donation and  $\alpha_1$ - or  $\alpha_2$ -AR antagonism, are currently being evaluated. The third direction is new targets within the central nervous system. Melanocortin receptor agonists have shown promise not only in animal models, but also in preliminary studies in humans. Other possible targets, such as growth hormone-releasing peptide receptors, are being explored. The fourth direction is new peripheral targets. Rho-kinase antagonism and non-nitric oxide-mediated stimulation of soluble guanylyl cyclase have been suggested as possible new principles for drug development. The fourth direction is gene therapy. Progress has been made in intracavernosal somatic gene therapy and will probably continue. Still, problems remain, and advantages over conventional pharmacological therapies have to be demonstrated. The final direction is prevention strategies. Strategies to prevent cavernosal degeneration and/or to restore cavernosal function will be one of the most exciting challenges for future research.

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**Keywords:** erectile dysfunction; phosphodiesterase inhibitors; combination therapy; gene therapy; prevention

## Introduction

The research activity within the field of erectile dysfunction (ED) has increased markedly during the last decade, and much new information has been obtained on both basic mechanisms and the clinical utility of different pharmacological principles.<sup>1,2</sup> After the successful introduction of sildenafil, peripheral targets have been in focus for drug development, and a second generation of phosphodiesterase (PDE) inhibitors is currently being introduced. However, the recent introduction of apomorphine as a treatment option has directed interest also to central mechanisms as targets for pharmacological interventions. Gene therapy based on introduction or overexpression of genes that can influence erection seems promising, and new information is continuously added. Based on results

of current activities, several directions for future research may be predicted, and the following directions appear promising and will be briefly discussed: (1) improvement of current therapeutic principles, (2) combinations of existing therapeutic principles, (3) new targets within the central nervous system, (4) new peripheral targets, (5) gene therapy, and (6) prevention strategies.

## Improvement of current therapeutic principles

### *New selective PDE inhibitors*

PDEs catalyze the hydrolysis of the second messengers cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), which are involved in signal pathways of cavernous smooth muscle. Because of their central role in smooth muscle tone regulation and the considerable variation of PDE isoenzymes with respect to species

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and tissues, PDEs have become an attractive target for drug development. The protein superfamily of cyclic nucleotide PDEs can be subdivided into at least 11 families of structurally and functionally related enzymes. More than 40 isoforms have been characterized so far, all differing in their primary structures, specificity for cAMP and cGMP, cofactor requirements, kinetic properties, mechanisms of regulation, and tissue distributions.<sup>3-5</sup> In human cavernous tissue, the presence of messenger RNA (mRNA) transcripts specific for 14 different human PDE isoenzymes and isoforms has been demonstrated by reverse transcription-polymerase chain reaction: three isogenes of PDE-1, PDE-2A, and PDE-10A, which hydrolyze cAMP and cGMP; the cAMP-specific PDE-3A; four isogenes of PDE-4, PDE-7A, and PDE-8A; and the cGMP-specific PDEs PDE-5A and PDE-9A.<sup>5</sup> PDE-5 constitutes the majority of the cGMP hydrolytic activity in corpora cavernosa smooth muscle cells, and functionally, PDE-3A and PDE-5A seem to be the most important.<sup>2,3</sup> Lin *et al*<sup>6</sup> reported cloning of three PDE-5 isoforms from human penile tissues. Two of the isoforms were identical to PDE-5A1 and PDE-5A2, respectively, which had previously been isolated from non-penile tissue. The third isoform, PDE-5A3, was novel; this isoform was confined to tissues with a smooth muscle or cardiac muscle component. PDE-5A3 may be an interesting target for future drug developments.

Second-generation, selective PDE-5 inhibitors, including vardenafil<sup>7</sup> and tadalafil (IC-351),<sup>8</sup> are about to be introduced into the market, and several other drugs with the same mode of action are in different stages of development.<sup>9-11</sup> It is expected that at least some of these oral agents will offer patients less adverse effects and versatility in rate of onset and duration of action. Currently, there are no cAMP PDE inhibitors available for the treatment of ED. However, the presence of both type 3 and type 4 PDE enzymes in the human corpus cavernosum opens the possibility that in the future, specific cAMP PDE inhibitors might be targets for the pharmacological treatment of ED.

#### *New dopamine receptor agonists*

It is well established that dopaminergic mechanisms are involved in the regulation of male sexual behavior in animals and humans. It has also been known for many years that apomorphine, a dopamine receptor agonist that stimulates both dopamine D<sub>1</sub> and D<sub>2</sub> receptors, can induce penile erection in rats and healthy and impotent men.<sup>12</sup> In rats, dopamine D<sub>2</sub> receptor stimulation appears to be responsible for the erectogenic effect, and this seems to be the case also in man.<sup>13</sup> Investigations involving low-dose systemic administration of sev-

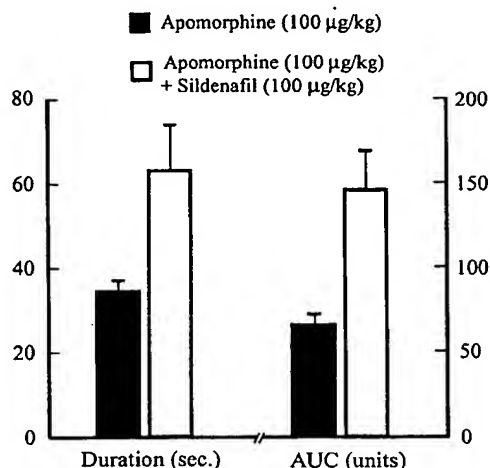
eral dopamine D<sub>2</sub> receptor agonists, such as piribedil, lisuride, and quinolorane, to rats and other animals (for review, see Andersson and Wagner<sup>12</sup>) have shown that the principle works. However, for different reasons, both pharmacodynamic (side effects) and pharmacokinetic, the development of ED drugs has been slow. Apomorphine, which is not effective orally and has a short duration of action, was found to have a limited therapeutic potential when injected, because of frequently occurring adverse effects, including emesis, yawning, drowsiness, transient nausea, lacrimation, flushing, and dizziness. However, Heaton and co-workers<sup>14</sup> demonstrated that apomorphine absorbed through the oral mucosa can act as an erectogenic agent with few adverse effects. Further extensive clinical experiences with sublingual apomorphine, 2 and 3 mg, have recently led to approval of apomorphine for clinical use in several countries, and available information suggests that sublingual apomorphine is an effective and reasonable alternative for patients with ED.<sup>15</sup> Still, apomorphine has a narrow therapeutic window, and further exploration of other dopamine receptor agonists may lead to as efficacious, but safer, alternatives.

#### *Combination of existing therapeutic principles*

The obvious aim of combination therapy is to increase efficacy and/or decrease adverse effects. The availability of efficacious oral treatments of ED with different modes of action makes it logical to try combination therapy in patients who respond unsatisfactorily to single-drug therapy. Combination of a centrally acting agent with a drug with a peripheral site of action seems attractive, but other combinations may also be interesting alternatives. However, it is important to underline that to really take advantage of combinations and to find optimal dosages for effective and safe therapy, randomized controlled clinical trials should be performed. Before such information has been obtained, combined use is not generally recommended.

#### *Apomorphine and sildenafil*

By acting on dopamine receptors on central nervous structures that control penile erection, apomorphine generates a signal that starts the erectile process in the target organ. If the responsiveness of the mechanisms that mediate erection in the penis is increased, a good erectile response should be expected. A combination of apomorphine and sildenafil would fulfill these requirements. Using a rat model, it was shown that an apomorphine-induced increase in intracavernous pressure could



**Figure 1** Sildenafil prolongs the erectile responses to apomorphine in rats. Data are from Andersson *et al.*<sup>16</sup>

be prolonged by sildenafil (Figure 1).<sup>16</sup> These results suggested that there may be a role for the combination of apomorphine and sildenafil in the management of ED.

### Apomorphine and $\alpha$ -adrenoceptor antagonists

Another combination that seems attractive is a central impulse generator (apomorphine) and a drug that decreases sympathetic tone in the penile erectile tissues. A necessary requirement is that the  $\alpha$ -adrenoceptor (AR) antagonist has no negative effects on cardiovascular functions. In human corpus cavernosum tissue, both  $\alpha_1$ - and  $\alpha_2$ -ARs have been demonstrated, but available information supports the view of a functional predominance of  $\alpha_1$ -ARs. This may be the case also in the penile vasculature, although an important contribution of  $\alpha_2$ -ARs to the contraction induced by noradrenaline (NA) and electrical stimulation of nerves cannot be excluded.<sup>17</sup> All the subtypes of  $\alpha_1$ -AR with high affinity for prazosin ( $\alpha_{1A}$ ,  $\alpha_{1B}$ , and  $\alpha_{1D}$ ) have been demonstrated in human corporal tissue; however, mRNAs for the  $\alpha_{1A}$ - and  $\alpha_{1D}$ -ARs are predominating. Functional studies of human corpus cavernosum tissue have suggested that the NA-induced contraction in this tissue may be mediated by several receptor subtypes.<sup>17</sup> An additional  $\alpha_1$ -AR subtype with low affinity for prazosin ( $\alpha_{1L}$ ), which has not been cloned, has been demonstrated in, for example, vascular smooth muscle. The possibility that the  $\alpha_{1L}$ -AR subtype, which may be a conformational state of the  $\alpha_{1A}$ -AR,<sup>18</sup> is of importance in human penile erectile tissues was recently suggested by Davis *et al.*,<sup>19</sup> who also found that the  $\alpha_{1D}$ -AR

selective compound BMY 7378 was inactive in human corpus cavernosum preparations. Using a selective antagonist of  $\alpha_{1A}$ -ARs, Ro70-0004/003, Choppin *et al.*<sup>20</sup> found that *in vitro* the drug effectively antagonized NA-induced contractions. In a randomized, placebo-controlled, crossover study of 24 patients with ED, this highly selective drug, given orally, did not improve erectile function compared with placebo.<sup>20</sup>

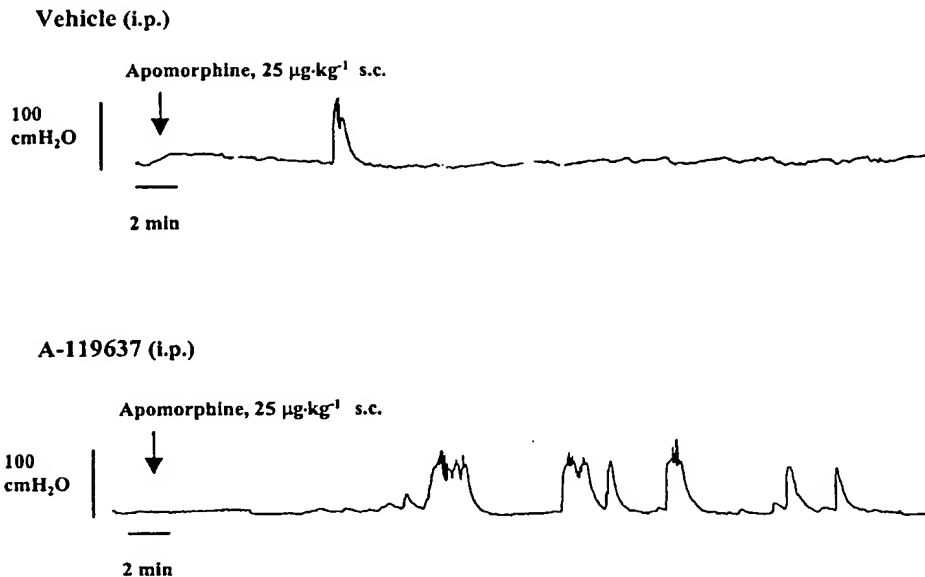
Experiments in animal models have given conflicting results. *In vivo* experiments in rats suggested that the  $\alpha_{1B}$ - and  $\alpha_{1L}$ -AR subtypes were functionally relevant for erectile function.<sup>21</sup> However, *in vitro*,  $\alpha_{1A}$ -ARs were found to be responsible for the contractile response of rat corpus cavernosum,<sup>22</sup> which does not seem to be in agreement with the *in vivo* data reported by Sironi *et al.*<sup>21</sup> In contrast to these results, Mizusawa *et al.* (unpublished data) found that in rats an  $\alpha_{1D}$ -AR antagonist proved to be effective both *in vitro* and *in vivo*. The combination of apomorphine and  $\alpha_{1D}$ -AR antagonism proved very effective (Mizusawa *et al.*, unpublished data; Figure 2). Whether the combination of apomorphine and a subtype selective  $\alpha$ -AR antagonist will be advantageous also in humans would be worth investigating.

### Nitrosylated $\alpha$ -AR antagonists

Thymoxamine (moxisylyte) and yohimbine are currently used for the pharmacological treatment of ED.<sup>1</sup> Moxisylyte has a competitive and relatively selective blocking action on  $\alpha_1$ -ARs, and *in vitro*, moxisylyte relaxed NA-contracted human corpus cavernosum preparations,<sup>23</sup> but was less potent than prazosin and phentolamine. Intracavernosal injection of moxisylyte has been shown to facilitate erection compared with placebo.<sup>24</sup>

Yohimbine has been used for many years as an oral agent for the treatment of ED. Although its effects on facilitating erection in animal models have been well demonstrated, its therapeutic value for the treatment of impotence in humans has been questioned.<sup>1</sup>

Nitrosylation of moxisylyte and yohimbine resulted in compounds (SNO-moxisylyte and SNO-yohimbine) that *in vitro* were significantly more potent than their parent compounds. *In vivo*, SNO-moxisylyte and SNO-yohimbine, given intracavernously, produced greater and more long-lasting increases in intracavernous pressure (rabbits) than the non-nitrosylated compounds, without having any effect on blood pressure.<sup>25</sup> It was suggested that SNO-moxisylyte and SNO-yohimbine may be useful therapeutic agents for the local (intracavernosal, transglandular, or transurethral) pharmacological treatment of ED.<sup>25</sup>



**Figure 2** The effects of apomorphine can be enhanced in the presence of  $\alpha_{1D}$ -adrenoceptor blockade in the rat. A-119637 is a selective  $\alpha_{1D}$ -adrenoceptor antagonist.<sup>69</sup> i.p. indicates intraperitoneally; s.c., subcutaneously.

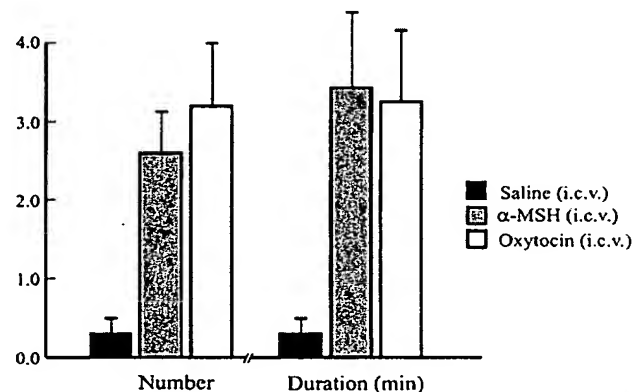
#### Other combinations

Angulo *et al*<sup>26</sup> analyzed the effects of combining the  $\alpha$ -AR antagonist phentolamine with L-arginine or sildenafil on nerve-induced relaxations in strips of the rabbit corpus cavernosum. They were able to demonstrate a synergistic interaction between the AR antagonist and the enhancers of the nitric oxide (NO)/cGMP pathway and suggested that the combination could represent a therapeutic advantage in the treatment of ED. Controlled clinical trials are required to prove or disprove this suggestion.

#### New targets within the central nervous system

##### Melanocortin receptors

After intracerebroventricular or hypothalamic periventricular injection into various animal models, the adrenocorticotrophic hormone (ACTH) and the  $\alpha$ -melanocyte stimulating hormone ( $\alpha$ -MSHs) induce penile erection (Figure 3) and ejaculation, grooming, stretching, and yawning.<sup>12,27</sup> Most, if not all, of the effects of the  $\alpha$ -MSH/ACTH peptides are mediated via specific subtypes of melanocortin (MC) receptors. Five different subtypes of MC receptor have been cloned and more or less extensively characterized.<sup>28,29</sup> The  $\alpha$ -MSH/ACTH peptides seem to act in the hypothalamic periventricular region, and grooming, stretching, and yawning, but not penile erection,



**Figure 3**  $\alpha$ -Melanocyte stimulating hormone (MSH) and oxytocin produce similar increases in number and duration of erectile responses in the rat when given intracerebroventricularly (i.c.v.).

tion, appear to be mediated by MC<sub>4</sub> receptors.<sup>27,30</sup> However, the subtype (or subtypes) associated with erectile responses has not been established.

Most of the information on proerectile effects of  $\alpha$ -MSH/ACTH peptides has been obtained in rats. However, the principle seems to work also in humans. Thus, Melanotan II, which is a cyclic nonselective MC receptor agonist, was found to be a potent initiator of penile erection in men with nonorganic ED, when injected subcutaneously.<sup>31–33</sup> However, yawning and stretching, and in some cases severe nausea and vomiting, limited its use. Nevertheless, the principle of MC receptor agonism,

maybe with subtype selective drugs, is a new and potentially useful therapeutic option.

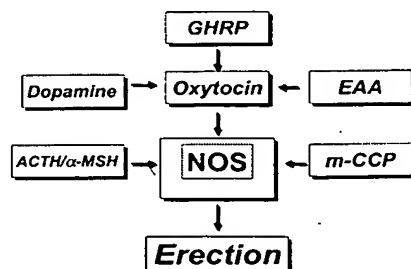
### Oxytocin and oxytocin receptors

When injected into the lateral cerebral ventricle, the paraventricular nucleus (PVN) of the hypothalamus, or the hippocampus of laboratory animals, oxytocin was found to be a potent inducer of penile erection.<sup>34-36</sup> The erectile response was blocked by oxytocin antagonists and by electrolytic lesion of the PVN.<sup>36-38</sup> The oxytocin-induced erections were also abolished by castration, and testosterone replacement restored erectile function.<sup>39</sup>

Several central neurotransmitters may converge on the oxytocinergic system as activators (for example, dopamine) or inhibitors (for example opioid peptides) of its transmission. Evidence supports calcium as a second messenger, mediating oxytocin-induced penile erection in the PVN and oxytocinergic receptor coupling with calcium channels through a pertussis toxin-sensitive G protein.<sup>40,41</sup> The oxytocinergic system may also be influenced by the NO synthase signal transduction pathway, since inhibitors of this pathway prevent penile erection and yawning in rats induced by oxytocin, dopamine, and *N*-methyl-D-aspartate (NMDA) stimulation.<sup>42-44</sup> Since many of the centrally active agents that can stimulate erection seem to act via oxytocinergic mechanisms (Figure 4), it would be logical to explore the potential of oxytocin and/or analogues in ED treatment.

### Growth hormone-releasing peptide receptors

A new class of peptide molecules that release growth hormone (GH) in experimental animals and humans, with an efficacy higher than that of the



**Figure 4** Many of the centrally active agents that can stimulate erection seem to act via oxytocinergic mechanisms. GHRP indicates growth hormone-releasing peptide; EAA, excitatory amino acid; CTH, adrenocorticotrophic hormone; MSH, melanocyte-stimulating hormone; and m-CCP, m-chlorophenylpiperazine, a 5-hydroxytryptamine<sub>2C</sub> receptor agonist.

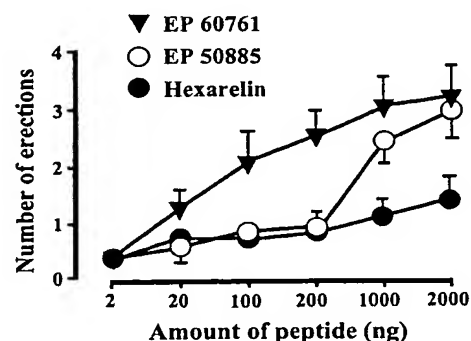
endogenous GH-releasing hormone (GHRH), has recently been characterized.<sup>45,46</sup> These GH-releasing peptides, like GHRH, also increase eating in laboratory animals when injected intracerebroventricularly, but the eating effect is not strictly related to the ability of these peptides to release GH,<sup>47</sup> since they act on receptors different from those activated by GHRH and are linked to intracellular messenger pathways different from those used by GHRH.<sup>46</sup>

In rats, EP 60761 and EP 50885, two analogues of the GH-releasing peptide hexarelin, were shown to induce penile erection episodes (Figure 5) indistinguishable from those elicited by dopamine receptor agonists, oxytocin, or NMDA when injected into the PVN and irrespective of their effect on GH release.<sup>48-50</sup> The results also suggested that EP 60761 and EP 50885 induced penile erection by increasing central oxytocin transmission, possibly by activating NO synthase in the cell bodies of oxytocinergic neurons located in the PVN that control penile erection.

### 5-Hydroxytryptamine receptors

It is well established that 5-hydroxytryptamine (5-HT; serotonin) neurons participate in the control of sexual behavior, both in humans and in animals. 5-HT pathways are considered to exert a general inhibitory effect on male sexual behavior.<sup>51</sup> However, these pathways may be inhibitory or facilitatory, depending on the action of the amine at different subtypes of 5-HT receptors located at different sites in the central nervous system.<sup>52</sup>

Many 5-HT receptor subtypes have been identified, which can rationally be divided into G protein-coupled and ligand-gated ion channel-related subfamilies.<sup>53</sup> The receptors use different effector systems in different cells, which may explain the conflicting reports on the effects of 5-HT agonists and antagonists on sexual functions. For example,



**Figure 5** Effects of hexarelin and analogues on erectile activity in rats. Data are from Melis *et al.*<sup>50</sup>

agonists may either enhance or depress sexual function, which has been attributed to the involvement of multiple 5-HT receptors. 5-HT<sub>1A</sub>, 5-HT<sub>1B</sub>, 5-HT<sub>2A</sub>, and 5-HT<sub>2C</sub> receptor subtypes have been found at different levels of the spinal cord.<sup>54-56</sup> In accordance with the selective use of 5-HT receptor agonists and antagonists, components of male copulatory behavior were found to be displayed variably. For example, 5-HT<sub>1A</sub> receptor activation may have contrasting effects on sexual function, depending on the dose of administration and location of the receptor in the brain.<sup>57</sup> Using immunohistochemistry, Bancila *et al*<sup>58</sup> found that the supraspinal serotonergic control of erection at the lumbosacral level appeared to be strongly associated with activation of 5-HT<sub>2C</sub> receptors. 1-(3-chlorophenyl)-piperazine, a trazodone metabolite, and *N*-trifluoromethylphenyl-piperazine are considered partial agonists at 5-HT<sub>2C</sub> receptors and usually display 5-HT<sub>2A</sub> receptor antagonistic actions.<sup>53</sup> They both induce erection in rodents, but they also significantly inhibit ejaculation and sexual behavior.<sup>2</sup> Several agonists with selective action on 5-HT<sub>2C</sub> receptors have been synthesized<sup>59</sup> and shown to induce penile erection in rats, suggesting an important role for the 5-HT<sub>2C</sub> receptor in the control of erectile mechanisms. Further supporting this, RSD 992, an agonist at 5-HT<sub>2C</sub> receptors, induced erections and facilitated male copulative behavior.<sup>60</sup>

Current developments of 5-HT<sub>2C</sub> receptor agonists may result in therapeutically useful drugs.

## New peripheral targets

### Guanylyl cyclases

Both membrane-bound (particulate) and soluble isoforms of guanylyl cyclases (GCs) are expressed in nearly all cell types.<sup>61</sup> Kim *et al*<sup>62</sup> demonstrated production of cGMP by particulate GC (GC-B) in rabbit and rat corpus cavernosum membranes stimulated by C-type natriuretic peptide 1-22

(CNP), atrial natriuretic peptide 1-28 (ANP), and brain natriuretic peptide 1-26. CNP but not ANP relaxed precontracted isolated preparations of rabbit corpus cavernosum.

Stief *et al*<sup>63</sup> demonstrated the mRNA expression of GC-B and the presence of the membrane-bound GC-B protein in the helical arteries of the human corpus cavernosum. In isolated cavernosal tissue, CNP induced a pronounced relaxation. Stief *et al*<sup>63</sup> speculated that this pathway may be a promising target for future ED treatment.

In the penis, soluble GC (sGC) is probably the most important receptor for NO as a signaling molecule. The enzyme, which catalyzes the conversion of guanosine triphosphate (GTP) into cGMP, consists of two different subunits and contains a prosthetic heme group. NO binds to this heme group and mediates up to 400-fold activation of the enzyme.<sup>61</sup>

YC-1, 3-(5'-hydroxymethyl-2'-furyl)-1-benzylindazole, was shown to elicit a direct activation of sGC by increasing the affinity for GTP and increasing the maximal enzyme activity, leading to increased cGMP levels in smooth muscle cells.<sup>64</sup> YC-1 also caused a large activation in the presence of the NO donor sodium nitroprusside, which lead to a remarkable 2200-fold stimulation of the human recombinant sGC.<sup>65</sup> In addition, YC-1 enhanced the sGC stimulating effect of carbon monoxide (31- to 34-fold above carbon monoxide alone).<sup>66</sup> Another NO-independent activator of sGC is BAY 41-2272,<sup>67</sup> which was reported to be 100-fold more potent than YC-1. YC-1 seems to be able to stimulate NO synthesis and release<sup>68</sup> and, in contrast to BAY 41-2272, to inhibit cGMP hydrolyzing PDEs,<sup>69</sup> enhancing the overall effect of cGMP.

YC-1 caused concentration-dependent relaxant responses in NA-contracted rat corpus cavernosum preparations and enhanced responses to electrical field stimulation.<sup>70,71</sup> YC-1 also enhanced the relaxant response induced by carbachol. *In vivo*, YC-1 elicited not only dose-dependent erectile responses when administered intracavernously, but also increased the effects on intracavernous pressure produced by stimulation of the cavernous nerve (Figure 6).

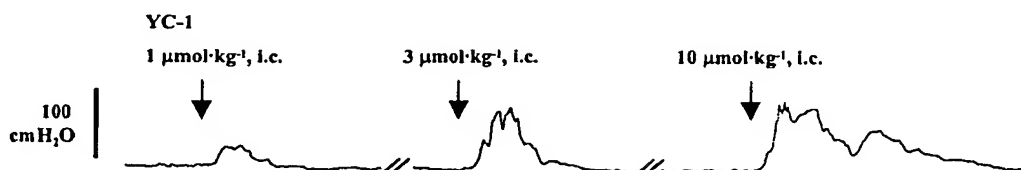


Figure 6 Dose-dependent erectile responses (intracavernous pressure) to intracorporeal (i.c.) injections of YC-1 in rats.



## Rho-kinase

In smooth muscle, the force/ $\text{Ca}^{2+}$  ratio is variable and depends partly on specific activation mechanisms. For example,  $\alpha$ -AR agonists induce a higher force/ $\text{Ca}^{2+}$  ratio than does a depolarization-induced increase (that is, potassium chloride) in intracellular  $\text{Ca}^{2+}$ , suggesting a 'Ca-sensitizing' effect of agonists. Furthermore, it has been shown that at a constant sarcoplasmic  $\text{Ca}^{2+}$  level, decrease of force (Ca desensitization) can be observed. The effect of Ca-sensitizing agonists is mediated by GTP-binding proteins that generate protein kinase C or arachidonic acid as second messengers.<sup>72,73</sup> The major mechanism of  $\text{Ca}^{2+}$  sensitization of smooth muscle contraction is through inhibition of the smooth muscle myosin phosphatase, thus increasing myosin light chain phosphorylation by basal-level activity of myosin light chain kinase. The resulting myosin phosphorylation and subsequent smooth muscle contraction therefore occur without a change in sarcoplasmic  $\text{Ca}^{2+}$  concentration.  $\text{Ca}^{2+}$  sensitization by the Rho-A/Rho-kinase pathway contributes to the tonic phase of the agonist-induced contraction in smooth muscle, and abnormally increased activation of myosin by this mechanism may play a role in certain diseases.<sup>74</sup> This calcium-sensitizing Rho-A/Rho-kinase pathway may also play a synergistic role in cavernosal vasoconstriction to maintain penile flaccidity. Rho-kinase is known to inhibit myosin light chain phosphatase and to directly phosphorylate myosin light chain, resulting in a net increase in activated myosin and the promotion of cellular contraction. Although Rho-kinase protein and mRNA have been detected in cavernosal tissue, the role of Rho-kinase in the regulation of cavernosal tone is unknown. Based on the hypothesis that antagonism of Rho-kinase results in increased corpus cavernosum pressure, initiating the erectile response independently of

NO, the effect of the Rho-kinase antagonist Y-27632 on cavernosal tone was examined.<sup>75,76</sup> The authors found that Y-27632 stimulated rat penile erection independently of NO (Figure 7) and suggested that the principle of Rho-kinase antagonism could be a potential alternative avenue for the treatment of ED.<sup>75</sup> Supporting this view, Rees *et al.*,<sup>77</sup> investigating the effect of Y-27632 on contractions induced by noradrenergic nerve stimulation and phenylephrine in human and rabbit corpus cavernosum, found that both types of contraction were inhibited by Y-27632 in a concentration-dependent way.

## Somatic gene therapy

Somatic gene therapy for ED, the genetic modification of differentiated cells, has produced encouraging preclinical results in various models of ED.<sup>1,78-80</sup> The penis seems to be a suitable organ for the use of gene therapy because of its external location, easy accessibility, and the low turnover rate of vascular smooth muscle cells, which will allow a desired gene to be expressed for long periods without affecting the systemic circulation. Gene therapy makes it possible to augment a missing or decreased relaxation-mediating component in the corpus cavernosum, and several approaches have been tried, including intracorporal injection of naked complementary DNA (cDNA) encoding inducible NO synthase, hSlo cDNA encoding the human smooth muscle maxi-potassium channel, and adenoviral gene transfer of endothelial NO synthase (Figures 8 and 9).<sup>81-84</sup> These studies support the view that *in vivo* gene transfer can have beneficial physiological effects on penile erection.

Whether or not gene therapy will represent the future of pharmacotherapy for ED is currently a matter of discussion. In this context, Moreland (personal communication) has raised the following interesting points. First, penile erection is a conditional response. The penis is erect for only a brief time during a 24-h period; however, most gene therapy constructs results in constitutive expression. Potentially, overexpression of NO synthase, for example, could result in prolonged erection and even priapism. Second, gene therapy requires redosing. If dosed frequently enough, what is the benefit of gene therapy over conventional on-demand pharmacologic treatment? Third, who is treatable? If cavernosal smooth muscle is damaged (cardiovascular disease, diabetes, hypercholesterolemia), will anything work? Fourth, what should research address? Research should select new and better targets and validate the current ones, such as potassium channels, NO synthase, vascular endothelial growth factor (VEGF), and neurotrophins. Better means of delivery need to be explored to maximize duration and efficiency of delivery. Genes

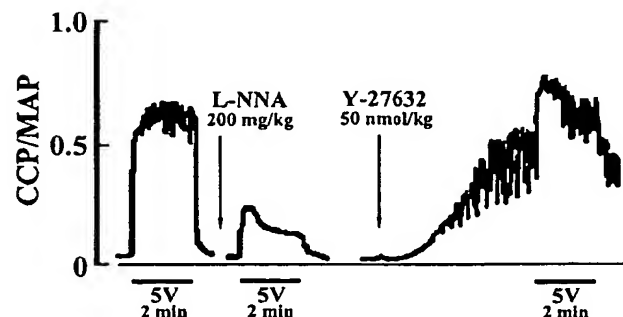


Figure 7 Nitric oxide-independent erectile response to the Rho-kinase antagonist Y-27632 in the rat. CCP indicates corpus cavernosum pressure; MAP, mean arterial pressure; and L-NNA, N<sup>G</sup>-nitro-L-arginine. Modified from Chitale *et al.*<sup>76</sup>

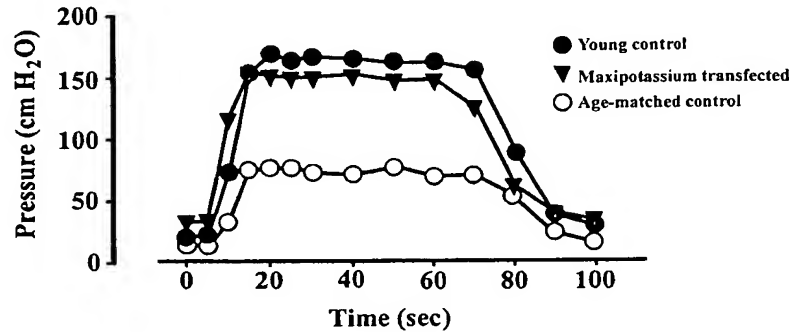


Figure 8 Effect (intracavernous pressure changes) of gene therapy with intracorporeally injected hSlo complementary DNA, encoding the human smooth muscle maxipotassium channel. Data are from Christ *et al.*<sup>82</sup>

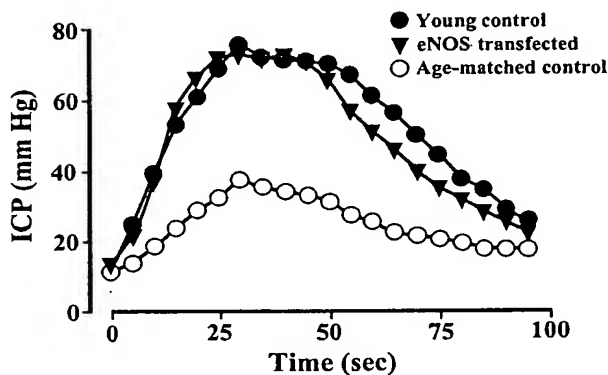


Figure 9 Effect (intracavernous pressure changes, ICP) of gene therapy with adenoviral gene transfer of endothelial nitric oxide synthase (eNOS) in rats. Data are from Champion *et al.*<sup>83</sup>

need to be identified and explored that work in combination with oral or injectable therapies. Is there a possibility of removing trabecular smooth muscle from the patient, culturing *in vitro*, transfecting a therapeutically beneficial gene, and reimplanting tissue-engineered corpus cavernosum?

Provided that future research will overcome some of these problems, gene therapy may develop into an effective therapeutic option.

## Prevention strategies

### Focus on risk factors and comorbidities

Previous studies that show associations between ED and heart disease, peripheral vascular disease, and stroke suggest that ED may be a sentinel event for underlying atherosclerosis.<sup>85</sup> ED shares several modifiable risk factors with vascular disease, in-

cluding cigarette smoking, hypertension, and unfavorable lipid levels. Obesity, sedentary behavior, and chronic alcoholism have also been implicated. The link between ED and vascular disease suggests that these risk factors may be promising targets for prevention. However, aging is probably the most important risk factor for ED, but it seems difficult to prevent. However, a common denominator for aging, diabetes, atherosclerosis, and smoking is a reduced efficacy of the NO/cGMP pathway, and ways of preventing or stopping progression of this reduction are desirable. In this context, the findings that intracavernosal injections of VEGF protect endothelium-dependent corpora cavernosal smooth muscle relaxation in hypercholesterolemic rabbits<sup>86,87</sup> is of great interest.

Derby *et al.*<sup>88</sup> investigated prospectively whether changes in smoking, heavy alcohol consumption, sedentary lifestyle, and obesity are associated with the risk of ED. They collected data as part of a cohort study of a random sample of men 40–70-y old selected from street listings in the Boston metropolitan area. In-home interviews were completed by 1709 men at baseline from 1987–1989 and 1156 men at follow-up from 1995–1997 (average follow-up, 8.8 y). Analyses included 593 men without ED at baseline, who were free of prostate cancer, and who had not been treated for heart disease or diabetes. It was concluded that midlife changes may be too late to reverse the effects of smoking, obesity, and alcohol consumption on ED. In contrast, physical activity may reduce the risk of ED, even if initiated in midlife. Derby *et al.* concluded that early adoption of healthy lifestyles may be the best approach to reducing the burden of ED on the health and wellbeing of older men.

## Conclusions

Ongoing research activities suggest that current ED therapies can and will be improved; there is a

rationale for combinations of existing oral therapies, but controlled clinical trials should be performed to optimize the potential advantages; new targets in the central nervous system and peripherally seem promising; gene therapy seems promising, but problems can slow development; and prevention strategies, including early adoption of a healthy lifestyle, should be implemented.

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## Appendix

### Open discussion following Dr Andersson's presentation

*Dr Nehra:* Can you shed some light on the functional significance of PDE-5A3, for example?

*Dr Andersson:* I only know that this isoform of PDE-5 has been identified in the human penis. I think we have to find out whether or not it is distributed all over the body before we attach any hope that inhibition of this particular enzyme would offer advantages over what we already have.

*Dr Porst:* To my knowledge, PDE-5A3 is very selective for the penis. It was only found in a small concentration in the cardiac system and nowhere else in the body.

*Dr Andersson:* It was found in the myocardium, which gives concern. That's why I am a little hesitant to say that this would solve any problem. This would maybe give an answer to whether or not organ specificity will be the final solution and the answer to what can be obtained by PDE-5 inhibition.

*Dr Porst:* Is there any place for endothelin antagonists in our armamentarium for developing drugs in the future or should we neglect the importance of the endothelins?

*Dr Andersson:* No, we shouldn't neglect the importance of the endothelins. We have been discussing now for 15 y endothelin and endothelin's role in penile erection. Since the initial observations that we could demonstrate endothelin generation in

penile erectile tissue, we have made no progress. We do not know whether or not endothelin is involved in erectile mechanism in a significant way. The reports on use of endothelin antagonists in erectile function or erectile mechanism have not given any clues as far as I know.

*Dr Montorsi:* Do we know if the currently available inhibitors of PDE-5 are inhibiting that subtype? And if we know that, are there any differences among sildenafil, tadalafil, and vardenafil with regards to the PDE-5A3 subtype?

*Dr Meuleman:* I'm aware of papers that show that ACE inhibition and cholesterol synthase inhibitors in the long run improve endothelial function, so these may be strategies to stop the process of vascular aging also in ED patients.

*Dr Andersson:* ACE inhibitors and also angiotensin II receptor blockers seem to be very useful, for example, in diabetes and renal vascular changes in diabetes. It's easy to extrapolate that this may also be a valid way to prevent vascular changes occurring in the penis. So far we have no information on that.

*Dr Giuliano:* Do you have any thought on the rationale for using chronic, daily dosing with PDE-5 inhibitors to prevent or act as a disease-modifying agent for erectile dysfunction?

*Dr Andersson:* I think that all therapies that are effective and can lead to maintaining sexual activity could have some improvement on erectile function and slow the progress of the disorder. This is one aspect of PDE-5 inhibitors. It may be slowing down the progress of the disease.